Title
Transforming growth factor-beta in breast cancer: too much, too late.

Journal
Breast cancer research : BCR, 11(1)

ISSN
1465-5411

Authors
Barcellos-Hoff, Mary Helen
Akhurst, Rosemary J

Publication Date
2009

DOI
10.1186/bcr2224

Peer reviewed

Review

Transforming growth factor-β in breast cancer: too much, too late

Mary Helen Barcellos-Hoff[1] and Rosemary J Akhurst[2]

[1]New York University Langone School of Medicine, New York, NY 10016, USA
[2]University of California, San Francisco, Comprehensive Cancer Center, San Francisco, CA 94143-0875, USA

Corresponding author: Mary Helen Barcellos-Hoff, Mhbarcellos-hoff@nyumc.org

Published: 26 February 2009
This article is online at http://breast-cancer-research.com/content/11/1/202

Breast Cancer Research 2009, **11:**202 (doi:10.1186/bcr2224)

Abstract

The contribution of transforming growth factor (TGF)β to breast cancer has been studied from a myriad perspectives since seminal studies more than two decades ago. Although the action of TGFβ as a canonical tumor suppressor in breast is without a doubt, there is compelling evidence that TGFβ is frequently subverted in a malignant plexus that drives breast cancer. New knowledge that TGFβ regulates the DNA damage response, which underlies cancer therapy, reveals another facet of TGFβ biology that impedes cancer control. Too much TGFβ, too late in cancer progression is the fundamental motivation for pharmaceutical inhibition.

Transforming growth factor-β in breast cancer progression

The breadth and scope of research to define the complex roles that transforming growth factor (TGF)β plays during mammary development and breast cancer now exceeds a thousand papers. Even by the time the elegant and oft-quoted study by Silberstein and Daniel in 1987 [1] put TGFβ on the mammary map as an important regulator of breast development, there was clear evidence that cancer could subvert this powerful growth inhibitory signal [2].

In the past decade or so, animal tumor studies that target over-expression or inactivation of various TGFβ signaling components to different epithelial compartments have resulted in a bewildering array of conclusions due to the pleiotropic and highly context-dependent action of TGFβ on cancer suppression or progression. It is now generally agreed that during early tumor outgrowth, elevated TGFβ is tumor suppressive, whereas at later stages there is a switch towards malignant conversion and progression [3,4], as shown in neu-induced mammary tumors [5]. Inactivation of tumor suppressor genes, the sequential acquisition of oncogenic mutations, and epigenetic changes within the cancer genome divert the canonical growth inhibitory arm of the TGFβ signaling pathway towards behaviors that increase motility, invasion and metastasis (reviewed in [4]). Consistent with the response to TGFβ evolving from growth inhibition to tumor progression during advanced malignancy, the majority of breast tumors, including their metastases, are positive for nuclear phosphorylated Smad2, indicating an actively signaling TGFβ pathway [6,7].

Loss of TGFβ growth inhibition and increased expression of TGFβ have been associated with malignant conversion and progression in breast, as well as gastric, endometrial, ovarian, and cervical cancers, glioma and melanoma (reviewed in [4,8]). But specific mutation of TGFβ signaling components occurs only occasionally in breast cancers. Rather, TGFβ growth response is abrogated by changes in the profile of other active signaling networks or the relative availability of transcriptional co-repressors or co-activators that bind to and modulate the canonical Smad pathway. Estrogens also appear to negatively regulate TGFβ signaling in breast cancer [9] and there is evidence that many pathway components may be epigenetically regulated during critical transitions in malignant progression [10].

TGFβ genetic predisposition to cancer

Genes encoding components of the TGFβ signaling pathway, including *TGFB1* [11], *TGFBRI* [12] and *TGFB2* [13], are functionally polymorphic in humans. *TGFB1* harbors promoter and signal peptide polymorphisms that influence protein secretion and levels of freely circulating TGFβ1 [11,14]. Several groups have demonstrated an association between variant *TGFB1* alleles and breast cancer risk [11,15,16]. The L10P allele increases protein production when expressed in culture and has been associated with high TGFβ levels [11]. The Breast Cancer Association Consortium conducted combined case-control analyses for breast cancer risk, and found odds ratios of 1.07 and 1.16 for L10P heterozygotes and homozygotes, respectively [17]. A case-control study of over 3,900 Caucasian women with early onset invasive breast cancer (median age 50 years) and a similar number of matched controls [11] demonstrated association between

EMT = epithelial to mesenchymal transition; FACS = fluorescent-activated cell sorting; TGF = transforming growth factor.

homozygosity for the high producer *TGFB1* L10P allele and an odds ratio of 1.25 for risk of invasive breast cancer. Similar associations have been found between hyperactive *TGFB1* variants and invasive prostate cancer [18], nasopharyngeal cancer [19], malignant melanoma [20], and lung cancer [21]. Conversely, a cohort study of more than 3,000 women aged 65 to 75 years suggested that homozygosity for hyperactive *TGFB1* appeared protective for breast cancer, suggesting that TGFβ1 has a breast tumor suppressing activity [15]. Pasche and colleagues [22] have proposed that hypomorphic variants of the TGFβ type I receptor interact with the hyperactive *TGFB1* variant to create 'high' versus 'low' signalers, the latter being associated with elevated breast cancer risk.

The disparate conclusions from these studies may be related to the age of the women and tumor grades in different studies. More recently, this apparent genetic dichotomy has been explained in terms of the dual function of TGFβ1 in carcinogenesis evident during neoplastic progression, as demonstrated in mouse models [3]. In a case control study of Asian breast cancer patients stratified according to tumor grade, hyperactive *TGFB1* was associated with decreased risk of early-stage breast cancer but increased risk of advanced breast cancer [23]. Given the complex biology regulated by TGFβ, there are probably other processes involved in mediating the TGFβ-associated risk of breast cancer. In different mouse strains, for example, homozygosity for a hypomorphic *Tgfb1* variant is genetically linked to skin tumor susceptibility. However, this effect can be completely masked by interacting genetic variants at a distant locus elsewhere in the genome [24]. It is likely that *Tgfb1* genotypes interact with other features in the genetic background [25].

Consequences of too much TGFβ

Elevated plasma TGFβ1 in hepatocellular carcinoma and breast, lung and prostate cancer patients correlates with poor outcome (reviewed in [26]). Systemic TGFβ1 levels have been used as a surrogate of tumor load and/or response to therapy [27,28]. Some circulating TGFβ1 may arise from the tumor; however, high plasma TGFβ1 levels can persist after tumor resection, suggesting that there may also be additional sources of the cytokine, such as blood cells, platelet degranulation or liver [29-31]. Compounding this, cancer therapy itself might induce TGFβ1 secretion by a number of routes (reviewed in [32-34]).

Epithelial to mesenchymal transition and the cancer stem cell

The tumor progressing activities of TGFβ are multifold, and involve effects on both the tumor cell and the tumor microenvironment [4]. It has been known for some time that TGFβ can induce epithelial to mesenchymal transition (EMT) in embryonic or neoplastic epithelial cells. This process is essential for normal embryonic development, and its exploitation during cancer progression has been thought to contribute to tumor invasion and metastasis [35]. In the mouse skin model of chemical carcinogenesis, overt EMT is a common occurrence, driven by TGFβ → Smad → Snail signaling, and resulting in the formation of highly aggressive, totally fibroblastic spindle carcinoma that have lost all the molecular markers of epithelial cells [3]. Radiation, a carcinogen of human breast, primes non-malignant human mammary epithelial cells to undergo TGFβ-mediated EMT [36]. Changes in motility elicited by cytoskeletal re-organization, and enhanced secretion of matrix-remodeling enzymes are classically considered the main driving forces in the contribution of reversible TGFβ-driven EMT to invasion and metastasis [37].

A recent paper from Polyak and colleagues [38] suggests an alternative mechanism. Expression profiling of fluorescent-activated cell sorting (FACS) sorted $CD44^{HIGH}$ $CD24^{LOW}$ marked cells, a population enriched for breast epithelial stem cells, showed transcripts associated with cell motility, cell adhesion, cell proliferation, chemotaxis and angiogenesis. The transcriptional similarity between FACS sorted populations enriched for normal and neoplastic stem cells was greater than that between them and the $CD44^{LOW}$ $CD24^{HIGH}$ population. The enrichment in transcripts for TGFβ and WNT signaling components was striking in these stem cells [38], suggesting preferential activation of these pathways and their functional involvement in stem cell biology. Indeed, putative stem cells were responsive to TGFβ and targeted by TGFβ inhibition, whereas the descendant $CD44^{LOW}$ $CD24^{HIGH}$ progenitor cells had lost responsiveness due to methylation of the *TGFBR2* gene. These data suggest that TGFβ signaling plays a role in mammary stem cell maintenance [38].

Taking this observation one step further, Mani and colleagues [39] showed that Snail-driven EMT in human mammary epithelial cells induces stem cell-like properties in terms of expression of stem cell markers, increased mammosphere seeding activity *in vitro* and tumorigenicity *in vivo*. Excessive TGFβ levels in the tumor microenvironment may, therefore, not only maintain putative cancer stem cells, but also contribute to their formation if more differentiated progenitors undergo EMT. This latter possibility remains to be tested. However, clinical evidence demonstrates that tumor expression of a 'TGFβ cassette' of genes (expressed in $CD44^{HIGH}$ $CD24^{LOW}$ > $CD44^{LOW}$ $CD24^{HIGH}$) is associated with shorter metastasis-free survival of patients with estrogen receptor-negative breast cancer [38]. These studies suggest that anti-TGFβ therapy could hold promise for targeting the cancer stem cell, especially within this TGFβ active sub-group of estrogen receptor-negative breast tumors.

Either as part of the stem cell 'phenotype' or independently of it, TGFβ can induce several other cell autonomous phenotypic changes that are conducive to tumor progression and metastasis. TGFβ signaling is clearly required for efficient

colonization of the lung by transformed cells [40], and expression of a TGFβ response expression signature in estrogen receptor-negative primary breast tumors is clinically associated with metastasis specifically to the lung but not to the bone [41]. One molecular mechanism responsible for this organ-specific tropism is TGFβ/Smad-driven activation of the gene encoding angiopoietin-like 4 (*ANGPTL4*). Angiopoietin-like 4 is a secreted ligand that disrupts tight endothelial barriers, such as those found in lung but not bone marrow, thus specifically stimulating pulmonary trans-endothelial migration of tumor cells [41]. Importantly, only transient exposure to TGFβ is required to induce the TGFβ response signature, which includes *ANGPTL4*, and to stimulate the consequent enhanced ability for lung colonization in a mouse metastasis model.

Tumor progression via microenvironment modification

Clearly, TGFβ has dramatic effects on epithelial phenotype, growth regulation and cell fate. Importantly, TGFβ has comparable control of the microenvironment composition mediated by effects on stromal, immune and vascular cells. Many investigators have argued that disruption of the stroma and tissue architecture can be a primary driver of carcinogenesis [42-46]. Recent experiments published from the labs of Weinberg [47], Moses [48], Sonnenschein [49] and Coussens [50] provide additional evidence that microenvironment composition is a critical determinant of cancer progression, which underscores the flipside of the cancer paradigm, that is, how the tissue becomes a tumor; TGFβ has a significant role on this side of the coin.

Tgfb1 null mice crossed onto an immune deficient background (which prevents neonatal death from gross inflammatory disease shortly after birth [51]) show little evidence of spontaneous cancer when housed under germ-free conditions. However, under standard mouse husbandry, these mice develop gastrointestinal cancer, supporting the concept that non-target cells mediate this epithelial tumorigenesis via TGFβ [52]. It is perhaps surprising to note that spontaneous cancer is not elevated in *Tgfb1* heterozygote mice up to 2 years, even though TGFβ production is severely compromised, even in Balb/C mice that are highly susceptible to breast cancer (MH Barcellos-Hoff and RJ Akhurst, unpublished data).

One of the major stromal targets for TGFβ action in tumor progression is the immune system. TGFβ acts in the tumor microenvironment to blunt immune-surveillance via multiple mechanisms, including suppression of both cytotoxic T and natural killer (NK) cells (reviewed in [53]). TGFβ recruitment of macrophages to the tumor also leads to a pro-inflammatory micro-environment, further exacerbating TGFβ production and the vicious cycle of tumor progression. Cell autonomous effects of TGFβ on the tumor cell provide protection from elimination by the immune system - for example, by down regulation of the expression of death receptors, major histocompatibility complex (MHC) molecules and Rae-1γ, the NKGD2 ligand required for NK cell activity. Recently, Wakefield and colleagues [54] demonstrated that TGFβ stimulates CD8+ T cells that infiltrate the tumor to produce interleukin-17, that in turn acts as a tumor cell survival factor via the interleukin-17 receptor.

These observations suggest that microenvironmental effects of TGFβ, together with its roles in EMT and metastasis, stimulate cancer progression and override any effects of TGFβ as a tumor suppressor in epithelia. These studies underscore the consensus opinion that TGFβ1 levels in cancer mediate a neoplastic plexus, driving cancer cells towards more aggressive behaviors and supporting their survival, while simultaneously limiting suppression by the host and perhaps augmenting normal tissue complications. The concept, put forward by Wakefield and colleagues [54], is that since excessive TGFβ action is mostly localized within the tumor, TGFβ inhibition could be therapeutically advantageous.

TGFβ, a malicious bystander during cancer therapy

TGFβ inhibition in either mouse or human mammary epithelial cells increases the cytotoxic response to ionizing radiation and several chemotherapeutic drugs [55-60]. Both radiation and chemotherapy induce TGFβ activity [61]. More importantly, Teicher and colleagues [62] showed that tumors secreting high levels of TGFß are more resistant to chemotherapy. Cis-platinum treatment of MDA-MB-231 breast cancer cells increased both TGFβ mRNA levels and the secretion of active TGFβ, which the authors suggest enhances growth arrest that facilitates repair of damage, thus rendering these cells resistant to cis-platinum killing [63]. Furthermore, treatment of MDA-MB-231 cells with anti-TGFß antibodies greatly enhanced cis-platinum-induced DNA fragmentation, augmented cell cycle progression and restored cellular sensitivity to cis-platinum [55]. Treatment of animals bearing cis-platinum-resistant tumors with TGFβ neutralizing antibody or with the TGFβ inhibitor decorin restored drug sensitivity of the tumor [56,57]. These authors suggested that inhibiting TGFβ-mediated cell cycle control would augment therapeutic efficacy.

Recent data suggest an even more proximal role for TGFβ in radiotherapy (reviewed in [64]). Breast cancer radiotherapy targets the tumor with the goal of inducing DNA damage resulting in cancer cell death, which increases long term patient survival [65]. Radiation-induced DNA damage elicits a signal transduction pathway that begins with sensor/activator proteins that lead to the activation of transducers that further convey the signal to multiple downstream effectors [66]. Recent studies have focused on ATM, a serine/threonine protein kinase required for the rapid response to radiation-induced DNA double strand breaks [67], as a means to amplify the therapeutic efficacy of radiation. Remarkably, the DNA damage response and subsequent cell fate decisions

are severely compromised if TGFβ is inhibited prior to irradiation in mouse epithelial tissues [59], human mammary epithelial cells [60,68] and lung cancer cells [60,68].

TGFβ depletion or signal inhibition does not affect ATM protein abundance, but actually blocks ATM kinase activity [60]. Both ATM autophosphorylation and phosphorylation of critical substrates, such as p53, Chk2 and Rad17, are abrogated, which in turn prevents cells from undergoing apoptosis or cell cycle arrest following DNA damage. As a consequence, epithelial cells are sensitized to radiation toxicity as assessed by clonogenic assays, just as if ATM is inhibited. Whether this potentially important therapeutic consequence will extend the use of TGFβ inhibitors in breast cancer treatment is unknown. Although a lung cancer cell line was rendered more resistant to radiation by use of small hairpin RNA inhibition of TGFβ receptors [68], preliminary studies using small molecule inhibition of TGFβ type I receptor kinase resulted in significant radiosensitization in four of five breast cancer cell lines (MH Barcellos-Hoff and A Pal, unpublished data). If TGFβ control of ATM is confirmed in tumors, then high tumor levels of TGFβ might actually amplify DNA damage signaling and repair, preventing tumor cell death, thereby limiting response to radiotherapy as Teicher and colleagues have shown for the response to chemotherapy [58].

Arteaga and colleagues [69] demonstrate that radiation-induced systemic TGFβ can also promote metastatic disease in breast cancer. In these studies, irradiated MMTV/PyVmT transgenic mice showed increased circulating levels of TGFβ1, circulating tumor cells, and lung metastases, which was abrogated by administration of a pan-neutralizing TGFβ antibody to the irradiated host. Hence, TGFβ inhibitors could block this tumor survival pathway and increase radiosensitivity, as well as preventing metastasis [69].

Radiotherapy-induced TGFβ activity is also implicated in late tissue toxicities that limit the use of radiotherapy for cancer treatment (reviewed in [32,33]. Normal tissues are spared from radio-toxicity in large part by physical targeting of tumors with conformal and targeted radiotherapy. Nonetheless, in some individuals, fibrosis can develop several years after therapy, which can affect quality of life or, in the case of lung tissue, be life-threatening. Unlike tumor control mediated by cell killing, fibrosis results from aberrant cytokine cascades principally initiated by TGFβ. Recent studies by Anscher and colleagues [33] have shown that even a single dose of anti-TGFβ antibody blocked radiation-induced lung injury, inflammatory response, and expression and activation of TGFβ from 6 weeks to 6 months after irradiation. Interestingly, EMT can contribute to fibrotic processes [70], and radiation appears to sensitize cells to TGFβ-mediated EMT [36].

These studies demonstrating that TGFβ activation is an undesirable side effect of radiotherapy provide further impetus for therapeutic inhibition. Along with the idea that TGFβ promotes breast cancer cell survival and metastasis at multiple levels, these data support the use of TGFβ inhibition during radiotherapy and chemotherapy. If effective, increased tumor response and decreased late tissue effects would result in a vastly improved therapeutic index for radiation treatment in breast cancer.

Future directions

The dysregulation of TGFβ in breast cancer, which in turn deregulates cellular and multicellular interactions to promote cancer, underlies one rationale for pharmaceutical TGFβ inhibition for breast cancer treatment. Immediate gain could be achieved by using TGFβ inhibitors to improve the response to chemo- and radiotherapy. Attenuation of undesirable effects, such as fibrosis, is yet another benefit of TGFβ inhibition, based on directly blocking processes that initiate pathology, or indirectly due to the anticipated reduction in radiation dose or scheduling necessary because of improved tumor response.

Concerns about limiting the activity of a growth factor whose action is essential to normal development and that plays crucial roles in wound healing and inflammation are valid but have yet to be confirmed in experimental cancer models. Perhaps, as suggested by several studies, the high levels of both protein and activity in the context of cancer elicit very different effects to those found in normal tissues where TGFβ activation is highly controlled. As proposed by Wakefield and colleagues [54], the 'locally distributed' activity may be the key to rational targeting. TGFβ inhibitors that reduce, rather than eliminate, TGFβ effects, used in combination with either targeted delivery to the tumor or a targeted therapy like radiation, may spare normal tissue at the expense of tumors (reviewed in [34]).

TGFβ-specific inhibitors based on blockade of synthesis, ligand/receptor binding or receptor kinase signaling are in clinical trials (reviewed in [53]). Pre-clinical models using TGFβ inhibitors have not yet elicited overt toxicity, and have shown efficacy by suppressing tumor metastasis, enhancing tumor responses to radio- and chemotherapy, and reducing normal tissue late effects. Given its complex biology, the biological target in breast cancer may be stromal, immune, vascular, or cancer stem cells, or all of these. Further research can refine the therapeutic rationale by focusing on drug scheduling and delivery, identifying patients who will benefit most from such therapy, and combining therapeutic modalities such that cancer is eliminated without normal tissue toxicity or long term health effects.

Competing interests

The authors declare that they have no competing interests.

Acknowledgements

The authors acknowledge funding from NIH RO-1 CA116019 (RKA) and the Department of Radiation Oncology of the NYU School of Medicine (MHBH).

References

1. Silberstein GB, Daniel CW: **Reversible inhibition of mammary gland growth by transforming growth factor-β.** *Science* 1987, **237:**291-293.
2. Basolo F, Fiore L, Ciardiello F, Calvo S, Fontanini G, Conaldi PG, Toniolo A: **Response of normal and oncogene-transformed human mammary epithelial cells to transforming growth factor beta 1 (TGF-beta 1): lack of growth-inhibitory effect on cells expressing the simian virus 40 large-T antigen.** *Int J Cancer* 1994, **56:**736-742.
3. Cui W, Fowlis DJ, Bryson S, Duffie E, Ireland H, Balmain A, Akhurst RJ: **TGFβ1 inhibits the formation of benign skin tumors, but enhances progression to invasive spindle carcinomas in transgenic mice.** *Cell* 1996, **86:**531-542.
4. Derynck R, Akhurst RJ, Balmain A: **TGF-β signaling in tumor suppression and cancer progression.** *Nat Genet* 2001, **29:**117-129.
5. Siegel PM, Shu W, Cardiff RD, Muller WJ, Massague J: **Transforming growth factor beta signaling impairs Neu-induced mammary tumorigenesis while promoting pulmonary metastasis.** *Proc Natl Acad Sci USA* 2003, **100:**8430-8435.
6. Xie W, Mertens JC, Reiss DJ, Rimm DL, Camp RL, Haffty BG, Reiss M: **Alterations of Smad signaling in human breast carcinoma are associated with poor outcome: A tissue microarray study.** *Cancer Res* 2002, **62:**497-505.
7. Kang Y, He W, Tulley S, Gupta GP, Serganova I, Chen C-R, Manova-Todorova K, Blasberg R, Gerald WL, Massague J: **Breast cancer bone metastasis mediated by the Smad tumor suppressor pathway.** *Proc Natl Acad Sci USA* 2005, **102:**13909-13914.
8. Schmierer B, Hill CS: **TGFbeta-SMAD signal transduction: molecular specificity and functional flexibility.** *Nat Rev Mol Cell Biol* 2007, **8:**970-982.
9. Kleuser B, Malek D, Gust R, Pertz HH, Potteck H: **17-beta-Estradiol inhibits transforming growth factor-beta signaling and function in breast cancer cells via activation of extracellular signal-regulated kinase through the G protein-coupled receptor 30.** *Mol Pharmacol* 2008, **74:**1533-1543.
10. Hinshelwood RA, Huschtscha LI, Melki J, Stirzaker C, Abdipranoto A, Vissel B, Ravasi T, Wells CA, Hume DA, Reddel RR, Clark SJ: **Concordant epigenetic silencing of transforming growth factor- signaling pathway genes occurs early in breast carcinogenesis.** *Cancer Res* 2007, **67:**11517-11527.
11. Pasche B, Kolachana P, Nafa K, Satagopan J, Chen YG, Lo RS, Brener D, Yang D, Kirstein L, Oddoux C, Ostrer H, Vineis P, Varesco L, Jhanwar S, Luzzatto L, Massagué J, Offit K: **TbetaR-I(6A) is a candidate tumor susceptibility allele.** *Cancer Res* 1999, **59:**5678-5682.
12. Beisner J, Buck MB, Fritz P, Dippon J, Schwab M, Brauch H, Zugmaier G, Pfizenmaier K, Knabbe C: **A novel functional polymorphism in the transforming growth factor-beta2 gene promoter and tumor progression in breast cancer.** *Cancer Res* 2006, **66:**7554-7561.
13. Cambien F, Ricard S, Troesch A, Mallet C, Generenaz L, Evans A, Arveiler D, Luc G, Ruidavets J-B, Poirier O: **Polymorphisms of the transforming growth factor-ß1 gene in relation to myocardial infarction and blood pressure: the Etude Cas-Temoin de l'Infarctus du Myocarde (ECTIM) Study.** *Hypertension* 1996, **28:**881-887.
14. Dunning AM, Ellis PD, McBride S, Kirschenlohr HL, Healey CS, Kemp PR, Luben RN, Chang-Claude J, Mannermaa A, Kataja V, Pharoah PD, Easton DF, Ponder BA, Metcalfe JC: **A transforming growth factorbeta1 signal peptide variant increases secretion *in vitro* and is associated with increased incidence of invasive breast cancer.** *Cancer Res* 2003, **63:**2610-2615.
15. Ziv E, Cauley J, Morin PA, Saiz R, Browner WS: **Association between the T29—>C polymorphism in the transforming growth factor beta1 gene and breast cancer among elderly white women: The study of osteoporotic fractures.** *JAMA* 2001, **285:**2859-2863.
16. Hishida A, Iwata H, Hamajima N, Matsuo K, Mizutani M, Iwase T, Miura S, Emi N, Hirose K, Tajima K: **Transforming growth factor B1 T29C polymorphism and breast cancer risk in Japanese women.** *Breast Cancer* 2003, **10:**63-69.
17. Cox A, Dunning AM, Garcia-Closas M, Balasubramanian S, Reed MW, Pooley KA, Scollen S, Baynes C, Ponder BA, Chanock S, Lissowska J, Brinton L, Peplonska B, Southey MC, Hopper JL, McCredie MR, Giles GG, Fletcher O, Johnson N, dos Santos Silva I, Gibson L, Bojesen SE, Nordestgaard BG, Axelsson CK, Torres D, Hamann U, Justenhoven C, Brauch H, Chang-Claude J, Kropp S, *et al.*: **A common coding variant in CASP8 is associated with breast cancer risk.** 2007, **39:**352-358.
18. Ewart-Toland A, Chan JM, Yuan J, Balmain A, Ma J: **A gain of function TGFB1 polymorphism may be associated with late stage prostate cancer.** *Cancer Epidemiol Biomarkers Prev* 2004, **13:**759-764.
19. Wei Y-S, Zhu Y-H, Du B, Yang Z-H, Liang W-B, Lv M-L, Kuang X-H, Tai S-H, Zhao Y, Zhang L: **Association of transforming growth factor-beta1 gene polymorphisms with genetic susceptibility to nasopharyngeal carcinoma.** *Clinica Chimica Acta* 2007, **380:**165-169.
20. Nikolova PN, Pawelec GP, Mihailova SM, Ivanova MI, Myhailova AP, Baltadjieva DN, Marinova DI, Ivanova SS, Naumova EJ: **Association of cytokine gene polymorphisms with malignant melanoma in Caucasian population.** *Cancer Immunol Immunother* 2007, **56:**371-379.
21. Kang HG, Chae MH, Park JM, Kim EJ, Park JH, Kam S, Cha SI, Kim CH, Park RW, Park SH, Kim YL, Kim IS, Jung TH, Park JY: **Polymorphisms in TGF-beta1 gene and the risk of lung cancer.** *Lung Cancer* 2006, **52:**1-7.
22. Kaklamani VG, Baddi L, Liu J, Rosman D, Phukan S, Bradley C, Hegarty C, McDaniel B, Rademaker A, Oddoux C, Ostrer H, Michel LS, Huang H, Chen Y, Ahsan H, Offit K, Pasche B: **Combined genetic assessment of transforming growth factor-beta signaling pathway variants may predict breast cancer risk.** *Cancer Res* 2005, **65:**3454-3461.
23. Shin A, Shu X-O, Cai Q, Gao Y-T, Zheng W: **Genetic polymorphisms of the transforming growth factor-beta1 gene and breast cancer risk: a possible dual role at different cancer stages.** *Cancer Epidemiol Biomarkers Prev* 2005, **14:**1567-1570.
24. Mao JH, Saunier EF, de Koning JP, McKinnon MM, Higgins MN, Nicklas K, Yang HT, Balmain A, Akhurst RJ: **Genetic variants of Tgfb1 act as context-dependent modifiers of mouse skin tumor susceptibility.** *Proc Natl Acad Sci USA* 2006, **103:**8125-8130.
25. Akhurst RJ: **TGF beta signaling in health and disease.** *Nat Genet* 2004 **36:**790-792.
26. Teicher BA: **Malignant cells, directors of the malignant process: role of transforming growth factor-beta.** *Cancer Metastasis Rev* 2001, **20:**133-143.
27. Anscher MS, Peters WP, Reisenbichler H, Petros WP, Jirtle RL: **Transforming growth factor β as a predictor of liver and lung fibrosis after autologous bone marrow transplantation for advanced breast cancer.** *New England J Med* 1993, **328:**1592-1598.
28. Kong F-M, Anscher MS, Murase T, Abbott BD, Iglehart JD, Jirtle RL: **Elevated plasma transforming gorwth factor-β1 levels in breast cancer patients decrease after surgical removal of tumor.** *Ann Surgery* 1995, **222:**155-162.
29. Tsushima H, Ito N, Tamura S, Matsuda Y, Inada M, Yabuuchi I, Imai Y, Nagashima R, Misawa H, Takeda H, Matsuzawa Y, Kawata S: **Circulating transforming growth factor beta1 as a predictor of liver metastasis after resection in colorectal cancer.** *Clin Cancer Res* 2001, **7:**1258-1262.
30. Barthelemy-Brichant N, David JL, Bosquée L, Bury T, Seidel L, Albert A, Bartsch P, Baugnet-Mahieu L, Deneufbourg JM: **Increased TGFbeta plasma level in patients with lung cancer: potential mechanisms.** *Europ J Clin Invest* 2002, **32:**193-198.
31. Shariat SF, Kattan MW, Traxel E, Andrews B, Zhu K, Wheeler TM, Slawin KM: **Association of pre- and postoperative plasma levels of transforming growth factor beta1 and interleukin 6 and its soluble receptor with prostate cancer progression.** *Clin Cancer Res* 2004, **10:**1992-1999.
32. Martin M, Lefaix J, Delanian S: **TGF-beta1 and radiation fibrosis: a master switch and a specific therapeutic target?** *Int J Radiat Oncol Biol Phys* 2000, **47:**277-290.
33. Anscher MS, Thrasher B, Rabbani Z, Teicher B, Vujaskovic Z: **Antitransforming growth factor-beta antibody 1D11 ameliorates normal tissue damage caused by high-dose radiation.** *Int J Radiat Oncol Biol Phys* 2006, **65:**876-881.
34. Erickson AC, Barcellos-Hoff MH: **The not-so innocent bystander: Microenvironment as a target of cancer therapy.** *Expert Opin Ther Targets* 2003, **7:**71-88.

35. Thiery JP: **Epithelial-mesenchymal transitions in development and pathologies.** *Curr Opin Cell Biol* 2003, **15:**740-746.
36. Andarawewa KL, Erickson AC, Chou WS, Costes SV, Gascard P, Mott JD, Bissell MJ, Barcellos-Hoff MH: **Ionizing radiation predisposes nonmalignant human mammary epithelial cells to undergo transforming growth factor beta induced epithelial to mesenchymal transition.** *Cancer Res* 2007, **67:**8662-8670.
37. Thiery JP: **Epithelial-mesenchymal transitions in tumour progression.** *Nat Rev Cancer* 2002, **2:**442-454.
38. Shipitsin M, Campbell LL, Argani P, Weremowicz S, Bloushtain-Qimron N, Yao J, Nikolskaya T, Serebryiskaya T, Beroukhim R, Hu M, Halushka MK, Sukumar S, Parker LM, Anderson KS, Harris LN, Garber JE, Richardson AL, Schnitt SJ, Nikolsky Y, Gelman RS, Polyak K: **Molecular definition of breast tumor heterogeneity.** *Cancer Cell* 2007, **11:**259-273.
39. Mani SA, Guo W, Liao MJ, Eaton EN, Ayyanan A, Zhou AY, Brooks M, Reinhard F, Zhang CC, Shipitsin M, Campbell LL, Polyak K, Brisken C, Yang J, Weinberg RA: **The epithelial-mesenchymal transition generates cells with properties of stem cells.** *Cell* 2008, **133:**704-715.
40. Pandey J, Umphress SM, Kang Y, Angdisen J, Naumova A, Mercer KL, Jacks T, Jakowlew SB: **Modulation of tumor induction and progression of oncogenic K-ras-positive tumors in the presence of TGF- 1 haploinsufficiency.** *Carcinogenesis* 2007, **28:** 2589-2596.
41. Padua D, Zhang XHF, Wang Q, Nadal C, Gerald WL, Gomis RR, Massagué J: **TGFbeta primes breast tumors for lung metastasis seeding through Angiopoietin-like 4.** *Cell* 2008, **133:**66-77.
42. Rubin H: **Cancer as a dynamic developmental disorder.** *Cancer Res* 1985, **45:**2935-2942.
43. Barcellos-Hoff MH: **The potential influence of radiation-induced microenvironments in neoplastic progression.** *J Mammary Gland Biol Neoplasia* 1998, **3:**165-175.
44. Sonnenschein C, Soto AM: **Somatic mutation theory of carcinogenesis: why it should be dropped and replaced.** *Mol Carcinog* 2000, **29:**205-211.
45. Bissell MJ, Radisky D: **Putting tumours in context.** *Nat Rev Cancer* 2001, **1:**1-11.
46. Wiseman BS, Werb Z: **Stromal effects on mammary gland development and breast cancer.** *Science* 2002, **296:**1046-1049.
47. Kuperwasser C, Chavarria T, Wu M, Magrane G, Gray JW, Carey L, Richardson A, Weinberg RA: **Reconstruction of functionally normal and malignant human breast tissues in mice.** *Proc Natl Acad Sci USA* 2004, **101:**4966-4971.
48. Bhowmick NA, Chytil A, Plieth D, Gorska AE, Dumont N, Shappell S, Washington MK, Neilson EG, Moses HL: **TGF-beta signaling in fibroblasts modulates the oncogenic potential of adjacent epithelia.** *Science* 2004, **303:**848-851.
49. Maffini MV, Soto AM, Calabro JM, Ucci AA, Sonnenschein C: **The stroma as a crucial target in rat mammary gland carcinogenesis.** *J Cell Sci* 2004, **117:**1495-1502.
50. de Visser KE, Eichten A, Coussens LM: **Paradoxical roles of the immune system during cancer development.** 2006, **6:**24-37.
51. Shull MM, Ormsby I, Kier AB, Pawlowski S, Diebold RJ, Yin M, Allen R, Sidman C, Proetzel G, Calvin D, *et al.*: **Targeted disruption of the mouse transforming growth factor-β1 gene results in multifocal inflammatory disease.** *Nature* 1992, **359:**693-699.
52. Engle SJ, Ormsby I, Pawlowski S, Boivin GP, Croft J, Balish E, Doetschman T: **Elimination of colon cancer in germ-free transforming growth factor beta 1-deficient mice.** *Cancer Res* 2002, **62:**6362-6366.
53. Saunier EF, Akhurst RJ: **TGF beta inhibition for cancer therapy.** *Curr Cancer Drug Targets* 2006, **6:**565-578.
54. Nam JS, Terabe M, Mamura M, Kang MJ, Chae H, Stuelten C, Kohn E, Tang B, Sabzevari H, Anver MR, Lawrence S, Danielpour D, Lonning S, Berzofsky JA, Wakefield LM: **An anti-transforming growth factor beta antibody suppresses metastasis via cooperative effects on multiple cell compartments.** *Cancer Res* 2008, **68:**3835-3843.
55. Ohmori T, Yang JL, Price JO, Arteaga CL: **Blockade of tumor cell transforming growth factor-betas enhances cell cycle progression and sensitizes human breast carcinoma cells to cytotoxic chemotherapy.** *Exp Cell Res* 1998, **245:**350-359.
56. Teicher BA, Holden SA, Ara G, Chen G: **Transforming growth factor-beta in *in vivo* resistance.** *Cancer Chemother Pharmacol* 1996, **37:**601-609.
57. Teicher BA, Ikebe M, Ara G, Keyes SR, Herbst RS: **Transforming growth factor-beta 1 overexpression produces drug resistance *in vivo*: reversal by decorin.** *In Vivo* 1997, **11:**463-472.
58. Liu P, Menon K, Alvarez E, Lu K, Teicher BA: **Transforming growth factor-beta and response to anticancer therapies in human liver and gastric tumors *in vitro* and *in vivo*.** *Int J Oncol* 2000, **16:**599-610.
59. Ewan KB, Henshall-Powell RL, Ravani SA, Pajares MJ, Arteaga CL, Warters RL, Akhurst RJ, Barcellos-Hoff MH: **Transforming growth factor-β1 mediates cellular response to DNA damage *in situ*.** *Cancer Res* 2002, **62:**5627-5631.
60. Kirshner J, Jobling MF, Pajares MJ, Ravani SA, Glick A, Lavin M, Koslov S, Shiloh Y, Barcellos-Hoff MH: **Inhibition of TGFβ1 signaling attenuates ATM activity in response to genotoxic stress.** *Cancer Res* 2006, **66:**10861-10868.
61. Kakeji Y, Maehara Y, Ikebe M, Teicher BA: **Dynamics of tumor oxygenation, CD31 staining and transforming growth factor-beta levels after treatment with radiation or cyclophosphamide in the rat 13762 mammary carcinoma.** *Int J Radiat Oncol Biol Phys* 1997, **37:**1115-1123.
62. Teicher BA, Maehara Y, Kakeji Y, Ara G, Keyes SR, Wong J, Herbst R: **Reversal of *in vivo* drug resistance by the transforming growth factor-beta inhibitor decorin.** *Int J Cancer* 1997, **71:** 49-58.
63. Hirohashi S, Kanai Y: **Cell adhesion system and human cancer morphogenesis.** *Cancer Sci* 2003, **94:**575-581.
64. Andarawewa KL, Kirshner J, Mott JD, Barcellos-Hoff MH: **TGFβ: roles in DNA damage responses.** In *Transforming Growth Factor-Beta in Cancer Therapy, Volume II Cancer Treatment and Therapy.* Edited by Jakowlew S. Totowa: Humana Press; 2007:321-334.
65. (EBCTCG) EBCTCG: **Effects of radiotherapy and of differences in the extent of surgery for early breast cancer on local recurrence and 15-year survival: an overview of the randomised trials.** *The Lancet* 2005, **366:**2087-2106.
66. Bakkenist CJ, Kastan MB: **Initiating cellular stress responses.** *Cell* 2004, **118:**9-17.
67. Shiloh Y: **ATM: Sounding the double-strand break alarm.** *Cold Spring Harb Symp Quant Biol* 2000, **65:**527-533.
68. Wiegman EM, Blaese MA, Loeffler H, Coppes RP, Rodemann HP: **TGFbeta-1 dependent fast stimulation of ATM and p53 phosphorylation following exposure to ionizing radiation does not involve TGFbeta-receptor I signalling.** *Radiother Oncol* 2007, **83:**289-295.
69. Biswas S, Guix M, Rinehart C, Dugger TC, Chytil A, Moses HL, Freeman ML, Arteaga CL: **Inhibition of TGF-beta with neutralizing antibodies prevents radiation-induced acceleration of metastatic cancer progression.** *J Clin Invest* 2007, **117:**1305-1313.
70. Kalluri R, Neilson EG: **Epithelial-mesenchymal transition and its implications for fibrosis.** *J Clin Invest* 2003, **112:**1776-1784.